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ESTIMATION OF THE LCt50 OF PHOSGENE IN SHEEP

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Inhalation of toxic doses of phosgene results in varying degrees of pulmonary edema, often after a symptom-free period, in all species studied. The sheep is an excellent animal in which to study the development of pulmonary edema by monitoring the effluent from a catheterized caudal mediastinal lymph node. In spite of this, there appear to be no published reports of sheep having ever been exposed to phosgene. This study was undertaken as a dose-ranging study in order to permit later studies in a phosgene-exposed sheep lung lymph preparation. Accordingly, the LCt50 (24 hours) was estimated by "up and down" subsequent dosage selection and by moving average interpolation methods.

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INTRODUCTION

The purpose of this study was to establish an estimate of the concentration of phosgene needed to cause death to 50% of sheep, within 24 hours after a ten-minute inhalation exposure (LCt50, 10 min).

Phosgene, a known chemical warfare agent, poses a serious problem in terms of medical chemical defense. Phosgene toxicity is also a public health and occupational lung disease problem. After inhalation exposure to toxic doses, the fluid-air barrier of the lung is breached and pulmonary edema develops. There is usually a latent period however, between the time of exposure and the appearance of clinical signs and symptoms [Diller, 1985].

The latent period for pulmonary edema can be studied experimentally in the laboratory with a well established sheep lung lymph preparation [Staub *et al*., 1975]. We have experience with this preparation and wished to compare physiologic data obtained with other pulmonary edemagenic compounds with data from phosgene-exposed sheep. To do this, we needed specific dosage information. A search of the literature revealed no reports of phosgene exposure in sheep under any conditions. We thus felt that a determination of the LCt50 of phosgene in sheep would be of general as well as of local interest to those concerned with the toxicology of phosgene.

MATERIALS AND METHODS

Animals:

Eight Dorset crossbred wethers (22-68 kg) were purchased from a purpose bred flock (Buckshire Corp., Perkasie, PA). Prior to delivery all sheep were tested and found negative for intradermal reaction to mammalian tuberculin and for serologic evidence of exposure to *Brucella ovis* and *Coxiella burnetii*. The sheep were segregated for 14 days during which they were examined, dewormed, and retested for exposure to *Coxiella burnetii*. Five to seven days before experimentation, the sheep were transferred from the holding pens to the laboratory and housed in pens at constant temperature (24°C) and on a 12-hour light/dark schedule. Tap water and a mixture of alfalfa hay and commercial pelleted ruminant feed (Zeigler Bros. Inc., Gardners, PA) were provided *ad libitum*. On the day of exposure the sheep were placed in ruminant metabolism/study cages (Research Equipment Co., Bryan, TX).

Experimental Paradigm:

Two sheep each were exposed to approximately 5,620, 10,000, 17,800, or 31,600 $mg \cdot min/m^3$ of phosgene over 10 minutes. Final observations of death or survival were made at 24 hours post-

exposure. The initial dosage, 10,000 mg·min/m^3, was selected based on lethal concentration data from goats [Diller and Zante, 1982]. Succeeding dosages (in 0.25 log increments) were selected based on the immediately preceding outcome. Necropsies were done 24 hours post-exposure on surviving sheep (after euthanasia) and at varying times post-exposure on dead sheep.

Dosage Parameters:

We selected a 10-minute exposure with 24-hour final observation for several reasons. There are no generally accepted standards for optimum exposure times and observation periods [Diller, 1982]. Ten-minute exposures, with observations carried out to 24 hours or slightly longer, were used in prior inhalation studies of organohalides [Jaeger et al., 1989]. We wanted to use similar times with phosgene to facilitate subsequent data comparisons. Choice of a 10-minute exposure permitted a reasonably short period of acute stress of the animal; however, it was sufficient exposure to avoid marked variations in amount of inhaled gas due to breath-holding. A 24-hour observation period is perhaps too short to encompass all deaths due to phosgene exposure, but it was chosen to provide optimum usage of personnel and facilities. Furthermore, postmortem specimens from euthanized surviving animals might show the subacute pathology of phosgene. Finally, both the 10-minute exposure period and the 24-hour observation period were frequently used in prior toxicology studies [Diller, 1982].

Inhalation Exposure Procedures:

Handling and administration of phosgene was done in an approved laboratory hood. The exposure system was designed to be used in either of three modes: exposure, purge or calibration. Compressed air (carrier gas for phosgene) flowed through two mass flow controllers (FC261, Tylan Co., Torrance, CA) that delivered a total of 15 l/min, monitored and controlled by a setpoint module (RO28, Tylan Co., Torrance, CA). Phosgene (Matheson Gas Co., Baltimore, MD), a compressed gas, was metered through another mass flow controller (MFC1259, Tylan Co., Torrance, CA) at a rate dependent on the desired concentration, monitored and controlled by another setpoint module (247B, MKS Instruments, Burlington, MA). In the calibration mode, a 4-way valve was turned to form a closed loop, a calculated amount of phosgene was injected via a gas-tight syringe, and the gas mixture was circulated by a pump (MB41, Metal Bellows, Sharon, MA) through an infrared spectrometer (Miran 1A, Foxboro Co., Foxboro, MA) to generate calibration curves according to procedures suggested in the Miran 1A instruction manual. Concentration data were recorded on a Kipp & Zonen BD 41 (Delft, Holland) chart recorder. In the exposure or purge mode, the 4-way valve was turned so that the gas mixture passed through the infrared spectrometer to monitor the delivered concentration, through the

passive circulating pump and then either directly to exhaust (purge mode) or to the sheep (exposure mode). Enroute to the sheep, the gas mixture passed through an anesthesia bag that served as a reservoir, through a 1-way valve, and then to the sheep via a cone sealed over the mouth and nose. Exhaled gas passed through another 1-way valve to the hood exhaust, which was filtered before being discharged to the environment.

Calculation of Phosgene Dosage:

Calibration dosages were calculated according to the Miran 1A instruction manual.

$$\text{Calibration Dosage} = \text{Desired Concentration of Phosgene} \times \frac{1}{\text{Density of Phosgene at ATPD}} \times \text{Volume of Calibration Loop}$$

$$\text{Example: } \frac{1\ \text{g}}{1000\ \text{l}} \times \frac{24\ \text{l}}{98.924\ \text{g}} \times 5.64\ \text{l} = 0.00137\ \text{l} = 1.37\ \text{ml}$$

LCt50 Estimation and Statistics:

Thompson and Weil analysis [Thompson, 1947; Weil, 1952] was used to estimate the LCt50 with 95% confidence limits. The requirements of the Thompson and Weil analysis are that 1) a constant number of animals be dosed at each dosage level with a minimum n=2, 2) at least four dosage levels be tested, and 3) the dosage levels be spaced so that the logarithms of successive dosages differ by a constant.

The equation for estimating the LCt50 is log LCt50 = log D + d(f + 1), where D is the lowest of the four dosage levels used, d is the log increment between successive doses, and f is a factor provided in tabular form in the published procedure of Weil [1952].

The equation for estimating a confidence interval that will encompass the LCt50 95 times in 100 is log LCt50 $\pm$ 2d(σ_f), where d is the log increment between successive dosages, and σ_f is the corresponding standard error of the f value.

Note: The table of f and corresponding σ_f values for n=2 and K=3 (dosage levels - 1) give a σ_f value of 0.00000, which is unreasonable; so instead the next highest σ_f value provided was chosen, and expressed as a lesser value, i.e., < 0.50000.

Necropsy and Histopathology:

Exposed sheep were submitted for necropsy 24 hours after exposure. Those sheep that died before this time point were

necropsied immediately at death or as soon thereafter as possible.

Euthanasia was accomplished by intravenous (jugular vein) injection of sodium pentobarbital (25 mg/kg) to induce a deep surgical plane of anesthesia followed by cervical and brachial exsanguination. Tissues were grossly examined and dissected, with particular attention to the respiratory tract. Tissue samples were routinely collected from each lung lobe, trachea, bronchial lymph nodes, and nasal turbinates, and immersion fixed in 10% neutral buffered formalin. Sections of lung were also fixed in formal Zenker's solution.

Following fixation, the tissues were dehydrated, embedded in paraffin, cut at five microns onto glass slides, and stained with hematoxylin and eosin. These sections were examined microscopically by a pathologist for qualitative interpretation.

RESULTS

LCt50:

Ten-minute exposures to phosgene resulted in the following data.

Experimental Day	Sheep I.D.#	Dose $mg \cdot min/m^3$	Dead at 24 hr	Comments
1	1119*	10000	no	dyspneic
2	1117*	31600	yes	dead 2 hr post-exposure
2	1113	31600	yes	dead 4 hr post-exposure
3	1115	10000	no	
4	1163	17800	yes	dead between 11
4	1151	17800	yes	and 19 hr post-exposure
5	1118	5620	no	
5	1159	5620	no	

* These two sheep were two weeks status post-thoracotomy and instrumented with lung lymph and vascular catheters for collection of physiologic data for another study.

The r-values for the tabulated data are 0,0,2,2 corresponding to the number of deaths at each of the dosage levels arrayed from smallest to largest.

Calculations:

$$\log LCt50 = \log 5620 + 0.25(0.50000 + 1)$$
$$= 3.74974 + 0.37500$$
$$= 4.12474$$
$$LCt50 = 13300 \text{ mg}\cdot\text{min/m}^3$$

$$95\% \text{ C.L.} = 4.12474 \pm 2(0.25)(< 0.50000)$$
$$= 4.12474 \pm < 0.25$$
$$\text{upper limit} = <4.37474; \text{ antilog is } <23700$$
$$\text{lower limit} = >3.87474; \text{ antilog is } >7490$$

Therefore, LCt50 (95% C.L.) = 13,300 (>7490 <23700) mg·min/m^3.

Gross and Histopathology:

All doses of phosgene induced some degree of pulmonary edema grossly and microscopically. At necropsy, severely edematous lungs did not collapse and had wet glistening pleural surfaces. Rib imprints were often clearly discernible on the diaphragmatic lobes. The lungs were extremely heavy and exuded clear fluid on cut surfaces. The airways extending from the proximal trachea to the smaller bronchioles were filled with a frothy white material. The tracheobronchial lymph nodes were mildly swollen. Less severely edematous lungs exhibited various congestion and anterior-ventral edema with distention of interlobular septa.

Microscopically, pulmonary edema ranged from being patchy and mild to diffuse and severe. The more severe edema was present in those sheep that received the higher doses and died less than 24 hours after exposure. The edema was characterized by filling of the alveoli, perivascular spaces and interlobular septa with a smooth eosinophilic exudate that occasionally contained strands of fibrin. Alveolar septa were thickened and mild numbers of macrophages were evident in alveoli. In several sheep, acute inflammatory lesions were evident with neutrophil infiltration present in alveoli, bronchioles, trachea, and/or the nasal cavity. Most of this inflammation was believed to be preexisting, although subsequent studies utilizing sham exposures are indicated to help resolve the significance of the acute inflammatory cell infiltrates. All sheep had some degree of peribronchial lymphocytic infiltrates indicative of previous immunologic stimulation. The sheep that had previous thoracic instrumentation exhibited extensive pleural fibrosis and underlying inflammatory lesions in the right lung lobes adjacent to the thoracotomy and lymphatic catheterization sites.

DISCUSSION

To the best of our knowledge, this is the first LCt50 data for phosgene in sheep. The use of a prior estimate in goats, a rough "up and down" subsequent dosage selection method, and the

moving average interpolation method of Thompson and Weil for calculating an LCt50 resulted in a minimum expenditure of animals.

Two of the sheep were instrumented with lymph and vascular catheters; however, this instrumentation did not appear to affect our results. Both the instrumented and uninstrumented sheep that were dosed at 31600 mg·min/m^3 died at approximately the same time (2 and 4 hours post-exposure, respectively). Microscopically, however, there were differences in lung tissue within each of the two instrumented-uninstrumented pairs of sheep: the instrumented sheep had relatively more edema than the uninstrumented sheep.

Pathologic examination of the tissues indicated a consistent induction of pulmonary edema at all exposure dosages which varied in clinical and pathologic severity according to dose and length of time following exposure.

As a result of this study, we have the information needed to induce varying degrees of injury by inhalation exposures to phosgene in sheep. In turn, the perturbed physiology and progression of pulmonary edema due to phosgene toxicity can be evaluated in an established sheep lung lymph preparation.

REFERENCES

Diller, W.F. and Zante, R.: Dosis-Wirkungs-Beziehungen bei Phosgen-Einwirkung auf Mensch und Tier. Zbl. Arbeitsmed. 32:360-368, 1982.

Diller, W.F.: Phosgene Induced Edema: Diagnosis and Therapeutic Countermeasures. In: Toxicology and Industrial Health Vol 1, #2, edited by M.A. Mehlman et al. Princeton Scientific Publishing Co., Inc., Princeton, 1985. Pp. 7-15.

Jaeger, J.J. et al.: manuscript in preparation.

Staub, N.C. et al.: Preparation of chronic lymph fistulas in sheep. J. Surg. Res. 19:315-320, 1975.

Thompson, W.R.: Use of moving averages and interpolation to estimate median-effective dose. Bacteriol. Rev. 11:115-145, 1947.

Weil, C.S.: Tables for convenient calculations of median effective dose (LD50 or ED50) and instructions in their use. Biometrics 8:249-263, 1952.

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